A PATHOGENIC LUMINESCENT BACTERIUM.

O. L. INMAN,

Antioch College.

In 1889 Giard and Billet (1) found that a number of different kinds of amphipod crustacea (*Talitrus*, *Orchestia*, *Ligia*, and others) gave off light. This luminosity was found to be caused by the presence of luminous bacteria in the tissues of the crustacea. In working with these luminous bacteria Giard and Billet were able to transfer them from one marine crustacean to another with ease and when grown on any marine crustacean tried, the bacteria became luminous. The authors could not get the bacteria to luminesce upon artificial media, although they obtained good growth. They offered no explanation for this failure and Harvey (2) pointed out that it seemed peculiar that these organisms could not be grown on artificial media so that they would produce light.

The luminous bacterium isolated by Giard and Billet is perhaps best known as *Bacterium Giardi* (Kruse) Mig., but much work is still needed upon the classification of luminous bacteria.

In August, 1925, several luminous individuals of *Orchestia platensis* (Kreyer) and *Talorchestia longicornus* (Say) were brought into the laboratory of E. N. Harvey at the Marine Biological Laboratory, Woods Hole, Massachusetts, and he turned them over to the author for investigation.

The sand flea chiefly studied was *Talorchestia longicornus* (Say) which can be found buried in the sand just above the high tide line during the daytime and feeding at the water's edge at night. This species is about the size of a honey bee and while they can be collected in June no luminous individuals have so far been found until August. These sand fleas will live several days in the laboratory if left in dishes of moist sand.

When luminous sand fleas were broken open and the grayish colored exudate mounted under the high power of a microscope,

numerous small motile rod-shaped bacteria could be seen. Inoculation of some of this material from the sand flea upon sea water peptone agar to which had been added sufficient sodium hydroxide to bring the pH to 8.1 resulted in the appearance of numerous bluish-green luminous colonies of bacteria in from twenty-four to thirty-six hours. These colonies were picked and pure cultures obtained. By a series of transfers it was readily determined that this bacterium in pure culture growing upon artificial media of many different kinds would produce light if the proper osmotic pressure and hydrogen ion concentration were maintained. The optimum, minimum, and maximum salt concentrations and hydrogen ion concentrations for growth and light production have not been determined but sufficient evidence is at hand to show that growth and light production may take place in a wide concentration of salts and hydrogen ion concentrations, the greater range from sea water being toward fresh water and neutrality. Data on this phase of the subject will be published in another paper.

Thus it at once became evident that Giard and Billet were unable to obtain luminous organisms upon artificial media because they failed to adjust the hydrogen ion concentration of their media which became neutral to acid when they added peptones to sea water and in such a case good growth may be obtained but no luminescence.

Giard and Billet also did sufficient experimentation to satisfy themselves that this bacterium was pathogenic to sand fleas. They inoculated sand fleas and followed the cases through to the death of the organisms which they claimed occurred more quickly than normal sand fleas will die in the laboratory.

The author repeated some of this work and found that it was possible to transfer the bacteria quite easily from one crustacean to another and that the administration of large numbers of this bacterium to a sand flea did cause it to die sooner than normal fleas. There were cases, however, when transfer of bacteria or feeding bacteria seemed to have doubtful effect and the sand fleas failed to become luminous. This led the author to collect non-luminous sand fleas from various habitats and isolate bacteria from the intestinal tract. In

almost every one of twenty separate sand fleas tried, luminous bacteria could be isolated and grew well and produced light on artificial media. The luminous bacteria isolated were quite surely *Bacterium Giardi*, but more proof is needed to demonstrate that this is the only species of luminous bacteria which inhabits the sand flea. This demonstrates, as pointed out by Dahlgren (3), that these bacteria are not necessarily luminous continuously, although it does not preclude the fact that they may be kept continually luminous without being passed through the sand flea, as has now been done for two years by the author. This observation also seems to point to the conclusion that this bacterium is a normal inhabitant of the body of the sand flea but that at certain times and under certain conditions it invades the muscles and increases in number so rapidly that the sand flea becomes luminous and dies. It is possible that the bacteria do not become luminous until they have spread to the muscular tissue of the sand flea and thus luminosity would mean that the host was doomed to die, since I have never observed a luminous sand flea recover. As to what causes the bacteria to invade the muscular tissue, especially in August at Woods Hole, Massachusetts, nothing yet is known. It may be a lowered resistance of the sand flea or increased reproductive activity of the bacterium due to a higher temperature or due to other causes. It should also be said that out of examination of possibly twenty thousand sand fleas only a very few ever become luminescent and it is quite possible that this bacterium plays a part in the death of thousands of sand fleas that never become luminescent. In other words, if this bacterium is really pathogenic, of which there is still some doubt, it is doubtful if there is any connection between luminosity and pathogenicity other than that great numbers of this bacterium under favorable environmental conditions naturally produce a maximum deleterious effect upon sand fleas and also give off more light.

Summary.

Amphipod crustacea are the host of *Bacterium Giardi* which becomes luminous under certain conditions and may kill the sand flea.

This bacterium, if isolated in pure culture and grown upon peptone sea water agar of pH 8.1, becomes luminous within twenty-four hours and may be kept so by frequent transfer for at least two years.

LITERATURE.

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